Rapid Double-Sandwich Enzyme-Linked Immunosorbent Assay for Detection of Human Immunoglobulin M Anti-Toxoplasma gondii Antibodies

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The double-sandwich enzyme-linked immunosorbent assay has been compared with the indirect fluorescence assay for the detection of immunoglobulin M antibodies against Toxoplasma gondii in humans. Incubation times have been shortened, permitting the test to be completed within 2 h. The double-sandwich enzyme-linked immunosorbent assay is confirmed to be more sensitive and more specific than the immunofluorescence assay.

We have recently reported on the use of whole Toxoplasma gondii parasites for the quantification of specific G and M antibodies by indirect enzyme-linked immunosorbent assay (ELISA) (8). This test has been favorably compared to the indirect immunofluorescence assay (IFA). However, the possibility of false-positive reactions due to rheumatoid factor (RF) has to be taken into account when the ELISA is applied to immunoglobulin M (IgM) determination (5, 6, 9, 10). The double-sandwich ELISA (DS ELISA) described for hepatitis A by Duermeyer and van der Veen (2) and adapted to toxoplasmosis by Naot and Remington (3, 4) has been shown to avoid any such interference. We have improved the procedure to allow completion of the test in 2 h.

Antigen. T. gondii tachyzoites were collected with sterile saline from the peritoneal exudate of mice infected 2 days earlier with the Rh strain, as previously described (7). The parasite suspension was frozen (−20°C) and thawed three times and centrifuged (2,000 × g) for 20 min at 4°C. The supernatant was collected and then filtered through a membrane filter (GSNP 02500; pore size, 0.22 μm; Millipore Corp., Bedford, Mass.). The lysate (3 mg of protein per ml) was stored at −20°C until later use.

Conjugate. Rabbit hyperimmune serum was obtained by subcutaneous inoculation of a brain homogenate from mice chronically infected with the Beverley strain of T. gondii (7). The IgG fraction was prepared by passing the serum through a Sepharose-protein A column (Pharmacia, Uppsala, Sweden). F(ab')2 obtained by pepsin digestion (2% [wt/wt]) was conjugated to horseradish peroxidase with glutaraldehyde by the two-step method (1). Conjugate purified by gel filtration on Ultrogel 44 (LKB, Bromma, Sweden) was used in the assay at a 1/200 dilution.

IgM DS ELISA. Wells of strips high binding capacity; (Nunc, Roskilde, Denmark) were coated with goat anti-human IgM antibodies (no. 4102; Tago, Burlingame, Calif.) diluted 1/1,000 in phosphate-buffered saline (pH 7.2) (PBS) by overnight incubation at 4°C (100 μl per well). Coated strips were washed three times with PBS, a procedure followed for all subsequent washing steps. Two hundred microliters of a 1/100 serum dilution in PBS supplemented with 0.5% (vol/vol) Tween 20 was incubated for 20 min at room temperature. The strips were washed again, 50 μl of antigen lysate diluted 1/2 in pure newborn calf serum (GIBCO, Paisley, Scotland) was added, and the strips were incubated for 30 min at 37°C and washed again. After the washing, 50 μl of conjugate, which was diluted with PBS containing 0.5% (vol/vol) Tween 20 and supplemented with 10% (vol/vol) newborn calf serum, was added, and the strips were incubated for 30 min at 37°C and washed again. After the final wash, substrate ABTS (Sigma Chemical Co., St. Louis, Mo.), at a concentration of 20 mg/100 ml of 0.05 M citrate–phosphate buffer (pH 4.0) containing 0.025% (vol/vol) hydrogen peroxide, was added (200 μl per well), and the strips were incubated for 30 min at 37°C in the dark. Optical densities were read at 414 nm.

One hundred four human sera, which were obtained from 80 patients, submitted routinely to the hospital, and suspected to contain IgM antibodies against T. gondii, were retained. Of these 104 sera, 19 contained RF, 13 contained antinuclear antibodies (ANA), and 8 contained both RF and ANA.

For as long as RF and ANA were absent from the sera tested, all specimens positive by IgM IFA were found to be positive by DS IgM ELISA (Fig. 1). However, in the same group of sera that were negative for both RF and ANA, DS IgM ELISA results were positive in 4 of 29 sera that were negative by IgM IFA. Serum no. 1 was obtained from an infant with proven congenital toxoplasmosis: T. gondii was isolated from the placenta (F. De Meuter, personal communication). Sera no. 2 and 3 both came from an infant who had cerebral calcifications and whose mother presented seroconversion to T. gondii during pregnancy. Serum no. 4 was obtained from the mother of the latter infant 1 month before delivery and 3 months after seroconversion. The comparison between DS IgM ELISA and IgM IFA results for sera containing RF or ANA or both is presented in Fig. 2. Of the 40 sera studied, 20 showed negative IgM IFA results. All of these 20 IFA-negative sera were also negative by DS IgM ELISA. On the other hand, 13 of 20 positive serum samples that were positive by IgM IFA were found to be negative by DS IgM ELISA. Among the 20 sera positive by IgM IFA, two deserve special comments. One of these (no. 5) was obtained from a woman with a recent history of acute toxoplasmosis confirmed by a rise of specific IgG

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FIG. 1. Correlation of IgM IFA and DS IgM ELISA in 64 RF- and ANA-free sera. Horizontal and vertical lines indicate cutoff values. Numbers enclosed in squares (sera no. 1 through 4) represent specimens mentioned in the text.

antibodies from negative to 1/8,000 by IgG IFA. The titer by IgM IFA was only 1/100, in contrast with the strongly positive result (optical density, 461% of cutoff value) obtained with DS IgM ELISA; this result confirms the higher sensitivity of the latter assay. The other serum (no. 6), which was obtained from a woman who had no suggestive symptoms of toxoplasmosis, was positive by IgM IFA (titer, 1/3,200) and negative by DS IgM ELISA; this patient presented both RF and ANA at very high levels (Waaler-Rose, 1/256; latex, 1/320; ANA, 1/640). These findings provide further proof for the specificity of DS IgM ELISA. Among the seven patients with sera positive by both IgM IFA and DS IgM ELISA, clinical data confirmed acute toxoplasmosis in four cases; unfortunately, no data were available in the other three cases.

Our results confirm that the use of the DS IgM ELISA for the detection and measurement of IgM specific antibodies will be an useful tool for the serodiagnosis of acute toxoplasmosis in humans. The retrospective diagnosis of two cases of congenital toxoplasmosis demonstrated the high sensitivity of the method. Furthermore, it is now possible to complete the whole test in less than 2 h, which is a great improvement as compared with previously described techniques. The method is therefore regarded as the best available routine procedure for the detection of T. gondii IgM antibodies.

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LITERATURE CITED

FIG. 2. Correlation of IgM IFA and DS IgM ELISA in 40 sera containing RF or ANA or both. Symbols: △, RF; ◇, ANA; *, RF and ANA. Horizontal and vertical lines indicate cutoff values. Numbers enclosed in squares (sera no. 5 and 6) represent specimens mentioned in the text.